(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 331 266 A1

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
30.07.2003 Bulletin 2003/31

(21) Application number: **01974718.7**

(22) Date of filing: **05.10.2001**

(51) Int Cl.[7]: **C12N 15/10**, C12P 21/08, C07K 16/00, A01K 67/00, A61K 39/395, C12N 9/00, C12N 15/52, G01N 33/53

(86) International application number:
PCT/JP01/08804

(87) International publication number:
WO 02/031140 (18.04.2002 Gazette 2002/16)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **06.10.2000 JP 2000308526**

(71) Applicant: **Kyowa Hakko Kogyo Co., Ltd**
Tokyo 100-8185 (JP)

(72) Inventors:
- **KANDA, Yutaka,**
  c/o Tokyo Research Laboratories
  Machida-shi, Tokyo 194-0023 (JP)
- **SATOH, Mitsuo,**
  c/o Tokyo Research Laboratories
  Machida-shi, Tokyo 194-0023 (JP)
- **NAKAMURA, Kazuyasu,**
  North Bethesda, MD 20852 (US)
- **UCHIDA, Kazuhisa,**
  c/o Tokyo Research Laboratories
  Machida-shi, Tokyo 194-0023 (JP)
- **SHINKAWA, Toyohide,**
  Tokyo Research Laboratories
  Machida-shi, Tokyo 194-0023 (JP)
- **YAMANE, Naoko,**
  c/o Tokyo Research Laboratories
  Machida-shi, Tokyo 194-0023 (JP)
- **HOSAKA, Emi, c/o Tokyo Research Laboratories**
  Machida-shi, Tokyo 194-0023 (JP)
- **YAMANO, Kazuya,**
  Tokyo Research Laboratories
  Machida-shi, Tokyo 194-8533 (JP)
- **YAMASAKI, Motoo,**
  c/o KYOWA HAKKO KOGYO CO.LTD
  Tokyo 1oo-8185 (JP)
- **HANAI, Nobuo,**
  c/o KYOWA HAKKO KOGYO CO,LTD
  Tokyo 100-8185 (JP)

(74) Representative: **VOSSIUS & PARTNER**
Siebertstrasse 4
81675 München (DE)

(54) CELLS PRODUCING ANTIBODY COMPOSITIONS

(57) The present invention relates to a cell for the production of an antibody molecule such as an antibody useful for various diseases having high antibody-dependent cell-mediated cytotoxic activity, a fragment of the antibody and a fusion protein having the Fc region of the antibody or the like, a process for producing an antibody composition using the cell, the antibody composition and use thereof.

EP 1 331 266 A1

bound to the Fc region in the composition, the ratio of a sugar chain in which fucose is not bound to *N*-acetylglucosamine in the reducing end in the sugar chain is 20% or more.

(2) The CHO cell according to (1), wherein the sugar chain to which fucose is not bound is a complex *N*-glycoside-linked sugar chain in which 1-position of fucose is not bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond.

(3) The CHO cell according to (1) or (2), wherein the antibody molecule belongs to an IgG class.

(4) The CHO cell according to any one of (1) to (3), wherein the activity of an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose and/or the activity of an enzyme relating to the modification of a sugar chain in which 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is decreased or deleted.

(5) The CHO cell according to (4), wherein the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose is an enzyme selected from the group consisting of the following (a), (b) and (c):

(a) GMD (GDP-mannose 4,6-dehydratase);
(b) Fx (GDP-keto-6-deoxymannose 3,5-epimerase, 4-reductase);
(c) GFPP (GDP-beta-L-fucose pyrophosphorylase).

(6) The CHO cell according to (5), wherein the GMD is a protein encoded by a DNA of the following (a) or (b):

(a) a DNA comprising the nucleotide sequence represented by SEQ ID NO:65;
(b) a DNA which hybridizes with the DNA comprising the nucleotide sequence represented by SEQ ID NO:65 under stringent conditions and encodes a protein having GMD activity.

(7) The CHO cell according to (5), wherein the GMD is a protein selected from the group consisting of the following (a), (b) and (c):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO:71;
(b) a protein which comprises an amino acid sequence in which at least one amino acid is deleted, substituted, inserted and/or added in the amino acid sequence represented by SEQ ID NO:71 and has GMD activity;
(c) a protein which comprises an amino acid sequence having a homology of at least 80% with the amino acid sequence represented by SEQ ID NO:71 and has GMD activity.

(8) The CHO cell according to (5), wherein the Fx is a protein encoded by a DNA of the following (a) or (b):

(a) a DNA comprising the nucleotide sequence represented by SEQ ID NO:48;
(b) a DNA which hybridizes with the DNA comprising the nucleotide sequence represented by SEQ ID NO:48 under stringent conditions and encodes a protein having Fx activity.

(9) The CHO cell according to (5), wherein the Fx is a protein selected from the group consisting of the following (a), (b) and (c):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO:72;
(b) a protein which comprises an amino acid sequence in which at least one amino acid is deleted, substituted, inserted and/or added in the amino acid sequence represented by SEQ ID NO:72 and has Fx activity;
(c) a protein which comprises an amino acid sequence having a homology of at least 80% with the amino acid sequence represented by SEQ ID NO:72 and has Fx activity.

(10) The CHO cell according to (5), wherein the GFPP is a protein encoded by a DNA of the following (a) or (b):

(a) a DNA comprising the nucleotide sequence represented by SEQ ID NO:51;
(b) a DNA which hybridizes with the DNA comprising the nucleotide sequence represented by SEQ ID NO:51 under stringent conditions and encodes a protein having GFPP activity.

(11) The CHO cell according to (5), wherein the GFPP is a protein selected from the group consisting of the following (a), (b) and (c):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO:73;
(b) a protein which comprises an amino acid sequence in which at least one amino acid is deleted, substituted,

inserted and/or added in the amino acid sequence represented by SEQ ID NO:73 and has GFPP activity;
(c) a protein which comprises an amino acid sequence having a homology of at least 80% with the amino acid sequence represented by SEQ ID NO:73 and has GFPP activity.

(12) The CHO cell according to (4), wherein the enzyme relating to the modification of a sugar chain in which 1-position of fucose is bound to 6-position of the *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is α-1,6-fucosyltransferase.
(13) The CHO cell according to (12), wherein the a-l,6-fucosyltransferase is a protein encoded by a DNA of the following (a) or (b):

(a) a DNA comprising the nucleotide sequence represented by SEQ ID NO:1;
(b) a DNA which hybridizes with the DNA comprising the nucleotide sequence represented by SEQ ID NO:1 under stringent conditions and encodes a protein having α-1,6-fucosyltransferase activity.

(14) The CHO cell according to (12), wherein the α-1,6-fucosyltransferase is a protein selected from the group consisting of the following (a), (b) and (c):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO:23;
(b) a protein which comprises an amino acid sequence in which at least one amino acid is deleted, substituted, inserted and/or added in the amino acid sequence represented by SEQ ID NO:23 and has α-1,6-fucosyltransferase activity;
(c) a protein which comprises an amino acid sequence having a homology of at least 80% with the amino acid sequence represented by SEQ ID NO:23 and has α-1,6-fucosyltransferase activity.

(15) The CHO cell according to any one of (4) to (14), wherein the enzyme activity is decreased or deleted by a technique selected from the group consisting of the following (a), (b), (c), (d) and (e):

(a) a gene disruption technique targeting a gene encoding the enzyme;
(b) a technique for introducing a dominant negative mutant of a gene encoding the enzyme;
(c) a technique for introducing mutation into the enzyme;
(d) a technique for inhibiting transcription or translation of a gene encoding the enzyme;
(e) a technique for selecting a cell line resistant to a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain.

(16) The CHO cell according to any one of (4) to (15), which is resistant to at least a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain.
(17) The CHO cell according to any one of (4) to (16), which produces an antibody composition having higher antibody-dependent cell-mediated cytotoxic activity than an antibody composition produced by its parent CHO cell.
(18) The CHO cell according to (17), which produces an antibody composition having higher antibody-dependent cell-mediated cytotoxic activity than an antibody composition in which among the total complex *N*-glycoside-linked sugar chains bound to the Fc region contained in the antibody composition, the ratio of a sugar chain in which fucose is not bound to *N*-acetylglucosarnine in the reducing end in the sugar chain is less than 20%.
(19) The CHO cell according to (18), wherein the sugar chain to which fucose is not bound is a complex *N*-glycoside-linked sugar chain in which 1-position of fucose is not bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond,
(20) A process for producing an antibody composition, which comprises culturing the CHO cell according to any one of (1) to (19) in a medium to produce and accumulate an antibody composition in the culture; and recovering the antibody composition from the culture.
(21) An antibody composition which is produced using the method according to (20).
(22) An antibody composition which comprises an antibody molecule having complex *N*-glycoside-linked sugar chains bound to the Fc region which is produced by a CHO cell, wherein among the total complex *N*-glycoside-linked sugar chains bound to the Fc region in the composition, the ratio of a sugar chain in which fucose is not bound to *N*-acetylglucosamine in the reducing end in the sugar chain is 20% or more.
(23) A cell in which the activity of an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose and/or the activity of an enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-

(a) a DNA comprising the nucleotide sequence represented by SEQ ID NO:1;
(b) a DNA comprising the nucleotide sequence represented by SEQ ID NO :2;
(c) a DNA which hybridizes with the DNA comprising the nucleotide sequence represented by SEQ ID NO:1 under stringent conditions and encodes a protein having α-1,6-fucosyltransferase activity,;
(d) a DNA which hybridizes with the DNA comprising the nucleotide sequence represented by SEQ ID NO:2 under stringent conditions and encodes a protein having α-1,6-fucosyltransferase activity.

(33) The cell according to (31), wherein the α-1,6-fucosyltransferase is a protein selected from the group consisting of the following (a), (b), (c), (d), (e) and (f):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO:23;
(b) a protein comprising the amino acid sequence represented by SEQ ID NO:24;
(c) a protein which comprises an amino acid sequence in which at least one amino acid is deleted, substituted, inserted and/or added in the amino acid sequence represented by SEQ ID NO:23 and has α-1,6-fucosyltransferase activity;
(d) a protein which comprises an amino acid sequence in which at least one amino acid is deleted, substituted, inserted and/or added in the amino acid sequence represented by SEQ ID NO:24 and has α-1,6-fucosyltransferase activity;
(e) a protein which comprises an amino acid sequence having a homology of at least 80% with the amino acid sequence represented by SEQ ID NO:23 and has α-1,6-fucosyltransferase activity;
(f) a protein which comprises an amino acid sequence having a homology of at least 80% with the amino acid sequence represented by SEQ ID NO:24 and has α-1,6-fucosyltransferase activity.

(34) The cell according to any one of (23) to (33), wherein the genetic engineering technique is a technique selected from the group consisting of the following (a), (b), (c) and (d):

(a) a gene disruption technique targeting a gene encoding the enzyme;
(b) a technique for introducing a dominant negative mutant of a gene encoding the enzyme;
(c) a technique for introducing mutation into the enzyme;
(d) a technique for inhibiting transcription and/or translation of a gene encoding the enzyme.

(35) The cell according to any one of (23) to (34), which is resistant to at least a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the *N*-glycoside-linked sugar chain.
(36) The cell according to any one of (23) to (35), which is a cell selected from the group consisting of the following (a) to (i):

(a) a CHO cell derived from a Chinese hamster ovary tissue;
(b) a rat myeloma cell line, YB2/3HL.P2.G11.16Ag.20 cell;
(c) a mouse myeloma cell line, NS0 cell;
(d) a mouse myeloma cell line, SP2/0-Ag14 cell;
(e) a BHK cell derived from a syrian hamster kidney tissue;
(f) an antibody-producing hybridoma cell;
(g) a human leukemia cell line, Namalwa cell;
(h) an embryonic stem cell;
(i) a fertilized egg cell.

(37) The cell according to any one of (23) to (36) into which a gene encoding an antibody molecule is introduced.
(38) The cell according to (37), wherein the antibody molecule belongs to an IgG class.
(39) A process for producing an antibody composition, which comprises culturing the cell according to (37) or (38) in a medium to produce and accumulate the antibody composition in the culture; and recovering the antibody composition from the culture.
(40) The process according to (39), which produces an antibody composition having higher antibody-dependent cell-mediated cytotoxic activity than an antibody composition obtained from its parent cell line.
(41) An antibody composition which is produced using the process according to (39) or (40).
(42) A transgenic non-human animal or plant or the progenies thereof, comprising a genome which is modified such that the activity of an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose and/or the activity of an enzyme relating to the modification of a sugar chain in which 1-position of fucose is bound to

inserted and/or added in the amino acid sequence represented by SEQ ID NO:73 and has GFPP activity;
(c) a protein which comprises an amino acid sequence having a homology of at least 80% with the amino acid sequence represented by SEQ ID NO:73 and has GFPP activity.

(12) The CHO cell according to (4), wherein the enzyme relating to the modification of a sugar chain in which 1-position of fucose is bound to 6-position of the *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is α-1,6-fucosyltransferase.
(13) The CHO cell according to (12), wherein the a-l,6-fucosyltransferase is a protein encoded by a DNA of the following (a) or (b):

(a) a DNA comprising the nucleotide sequence represented by SEQ ID NO:1;
(b) a DNA which hybridizes with the DNA comprising the nucleotide sequence represented by SEQ ID NO:1 under stringent conditions and encodes a protein having α-1,6-fucosyltransferase activity.

(14) The CHO cell according to (12), wherein the α-1,6-fucosyltransferase is a protein selected from the group consisting of the following (a), (b) and (c):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO:23;
(b) a protein which comprises an amino acid sequence in which at least one amino acid is deleted, substituted, inserted and/or added in the amino acid sequence represented by SEQ ID NO:23 and has α-1,6-fucosyltransferase activity;
(c) a protein which comprises an amino acid sequence having a homology of at least 80% with the amino acid sequence represented by SEQ ID NO:23 and has α-1,6-fucosyltransferase activity.

(15) The CHO cell according to any one of (4) to (14), wherein the enzyme activity is decreased or deleted by a technique selected from the group consisting of the following (a), (b), (c), (d) and (e):

(a) a gene disruption technique targeting a gene encoding the enzyme;
(b) a technique for introducing a dominant negative mutant of a gene encoding the enzyme;
(c) a technique for introducing mutation into the enzyme;
(d) a technique for inhibiting transcription or translation of a gene encoding the enzyme;
(e) a technique for selecting a cell line resistant to a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain.

(16) The CHO cell according to any one of (4) to (15), which is resistant to at least a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain.
(17) The CHO cell according to any one of (4) to (16), which produces an antibody composition having higher antibody-dependent cell-mediated cytotoxic activity than an antibody composition produced by its parent CHO cell.
(18) The CHO cell according to (17), which produces an antibody composition having higher antibody-dependent cell-mediated cytotoxic activity than an antibody composition in which among the total complex *N*-glycoside-linked sugar chains bound to the Fc region contained in the antibody composition, the ratio of a sugar chain in which fucose is not bound to *N*-acetylglucosarnine in the reducing end in the sugar chain is less than 20%.
(19) The CHO cell according to (18), wherein the sugar chain to which fucose is not bound is a complex *N*-glycoside-linked sugar chain in which 1-position of fucose is not bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond,
(20) A process for producing an antibody composition, which comprises culturing the CHO cell according to any one of (1) to (19) in a medium to produce and accumulate an antibody composition in the culture; and recovering the antibody composition from the culture.
(21) An antibody composition which is produced using the method according to (20).
(22) An antibody composition which comprises an antibody molecule having complex *N*-glycoside-linked sugar chains bound to the Fc region which is produced by a CHO cell, wherein among the total complex *N*-glycoside-linked sugar chains bound to the Fc region in the composition, the ratio of a sugar chain in which fucose is not bound to *N*-acetylglucosamine in the reducing end in the sugar chain is 20% or more.
(23) A cell in which the activity of an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose and/or the activity of an enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-

relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain.

[0120] The antisense oligonucleotide or ribozyme can be prepared in the usual method or using a DNA synthesizer. Specifically, it can be prepared based on the sequence information of an oligonucleotide having a corresponding sequence of continued 5 to 150 bases, preferably 5 to 60 bases, and more preferably 10 to 40 bases, among nucleotide sequences of a cDNA and a genome DNA encoding the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain, by synthesizing an oligonucleotide which corresponds to a sequence complementary to the oligonucleotide (antisense oligonucleotide) or a ribozyme comprising the oligonucleotide sequence.

[0121] Examples of the oligonucleotide include oligo RNA and derivatives of the oligonucleotide (hereinafter referred to as "oligonucleotide derivatives").

[0122] Examples of the oligonucleotide derivatives include oligonucleotide derivatives in which a phosphodiester bond in the oligonucleotide is converted into a phosphorothioate bond, an oligonucleotide derivative in which a phosphodiester bond in the oligonucleotide is converted into an N3'-P5' phosphoamidate bond, an oligonucleotide derivative in which ribose and a phosphodiester bond in the oligonucleotide are converted into a peptide-nucleic acid bond, an oligonucleotide derivative in which uracil in the oligonucleotide is substituted with C-5 propynyluracil, an oligonucleotide derivative in which uracil in the oligonucleotide is substituted with C-5 thiazoleuracil, an oligonucleotide derivative in which cytosine in the oligonucleotide is substituted with C-5 propynylcytosine, an oligonucleotide derivative in which cytosine in the oligonucleotide is substituted with phenoxazine-modified cytosine, an oligonucleotide derivative in which ribose in the oligonucleotide is substituted with 2'-*O*-propylribose and an oligonucleotide derivative in which ribose in the oligonucleotide is substituted with 2'-methoxyethoxyribose [*Cell Technology*, 16, 1463 (1997)].

(b) Preparation of the host cell of the present invention by homologous recombination

[0123] The host cell of the present invention can be produced by modifying a target gene on chromosome through a homologous recombination technique, using a gene encoding an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or an enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain as the target gene.

[0124] The target gene on the chromosome can be modified by using a method described in *Manipulating the Mouse Embryo, A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press (1994) (hereinafter referred to as "*Manipulating the Mouse Embryo, A Laboratory Manual*"); *Gene Targeting, A Practical Approach*, IRL Press at Oxford University Press (1993); *Biomanual Series 8, Gene Targeting, Preparation of Mutant Mice using ES Cells*, Yodosha (1995) (hereinafter referred to as "*Preparation of Mutant Mice using ES Cells*"); or the like, for example, as follows.

[0125] A genome DNA encoding an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or an enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is prepared.

[0126] Based on the nucleotide sequence of the genome DNA, a target vector is prepared for homologous recombination of a target gene to be modified (e.g., structural gene of the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain, or a promoter gene).

[0127] The host cell of the present invention can be produced by introducing the prepared target vector into a host cell and selecting a cell in which homologous recombination occurred between the target gene and target vector.

[0128] As the host cell, any cell such as yeast, animal cell, insect cell or plant cell can be used, so long as it has a gene encoding the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain. Examples include the host cells which will be described later in the item 3.

[0129] Examples of the method for preparing a genome DNA encoding the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain include the methods described in the preparation of genome DNA in the item 1(1)(a) and the like.

[0130] Examples of the nucleotide sequence of genome DNA encoding the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose include the nucleotide sequence represented by SEQ ID NO:67 or 70. Examples of the nucleotide sequence of genome DNA encoding the enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond

which 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the *N*-glycoside-linked sugar chain. Examples include a *Lens culinaris* lectin LCA (lentil agglutinin derived from *Lens culinaris*), a pea lectin PSA (pea lectin derived from *Pisum sativum*), a broad bean lectin VFA (agglutinin derived from *Vicia faba*), an *Aleuria aurantia* lectin AAL (lectin derived from *Aleuria aurantia*) and the like.

[0195] Specifically, the cell line of the present invention resistant to a lectin which recognizes a sugar chain structure in which 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the *N*-glycoside-linked sugar chain can be selected by culturing cells for 1 day to 2 weeks, preferably from 1 day to 1 week, using a medium comprising the lectin at a concentration of 1 μg/ml to 1 mg/ml, subculturing surviving cells or picking up a colony and transferring it into a culture vessel, and subsequently continuing the culturing using the lectin-containing medium. Examples of the cell line obtained by the method include CHO/CCR4-LCA Nega-13 (FERM BP-7756) obtained in Example 14(2) which will be described later.

2. Preparation of a transgenic non-human animal or plant or the progenies thereof of the present invention

[0196] The transgenic non-human animal or plant or the progenies thereof of the present invention is a transgenic non-human animal or plant or the progenies thereof in which a genome gene is modified in such a manner that the activity of an enzyme relating to the modification of a sugar chain of an antibody molecule can be controlled, and it can be prepared according to the method similar to that in the item 1, using a gene encoding an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or an enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain, as the target.

[0197] In a transgenic non-human animal, the embryonic stem cell of the present invention in which the activity of the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the activity of the enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is controlled can be prepared applying the method similar to that in the item 1 to an embryonic stem cell of the intended non-human animal such as cattle, sheep, goat, pig, horse, mouse, rat, fowl, monkey, rabbit or the like.

[0198] Specifically, a mutant clone is prepared in which a gene encoding the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is inactivated or substituted with any sequence, by a known homologous recombination technique [e.g., *Nature*, 326, 6110, 295 (1987); *Cell*, 51, 3, 503 (1987); or the like]. Using the prepared mutant clone, a chimeric individual comprising an embryonic stem cell clone and a normal cell can be prepared by an injection chimera method into blastocyst of fertilized egg of an animal or by an aggregation chimera method. The chimeric individual is crossed with a normal individual, so that a transgenic non-human animal in which the activity of the enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the activity of the enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is decreased or deleted in the whole body cells can be obtained.

[0199] Also, a fertilized egg cell of the present invention in which the activity of an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the activity of an enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is decreased or deleted can be prepared by applying the method similar to that in the item 1 to fertilized egg of a non-human animal of interest such as cattle, sheep, goat, pig, horse, mouse, rat, fowl, monkey, rabbit or the like.

[0200] A transgenic non-human animal in which the activity of an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the activity of an enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is decreased can be prepared by transplanting the prepared fertilized egg cell into the oviduct or uterus of a pseudopregnant female using the embryo transplantation method described in *Manipulating Mouse Embryo*, Second Edition or the like, followed by childbirth by the animal.

[0201] In a transgenic plant, the callus of the present invention in which the activity of an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the activity of an enzyme relating to the modification of a sugar chain wherein 1-position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked sugar chain is decreased or deleted can be prepared by applying the method similar to that in the item 1 to a callus or cell of the plant of interest.

[0202] A transgenic plant in which the activity of an enzyme relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose or the activity of an enzyme relating to the modification of a sugar chain wherein 1 -position of fucose is bound to 6-position of *N*-acetylglucosamine in the reducing end through α-bond in the complex *N*-glycoside-linked

sugar chain is decreased can be prepared by culturing the prepared callus using a medium comprising auxin and cytokinin to redifferentite it in accordance with a known method *[Tissue Culture,* 20 (1994); *Tissue Culture,* 21 (1995); *Trends in Biotechnology,* 15, 45 (1997)].

3. Process for producing an antibody composition

[0203] The antibody composition can be obtained by expressing it in a host cell using the methods described in *Molecular Cloning,* Second Edition; *Current Protocols in Molecular Biology; Antibodies, A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988 (hereinafter referred also to as "*Antibodies*"); *Monoclonal Antibodies: Principles and Practice,* Third Edition, Acad. Press, 1993 (hereinafter referred also to as *"Monoclonal Antibodies");* and *Antibody Engineering, A Practical Approach*, IRL Press at Oxford University Press (hereinafter referred also to as "*Antibody Engineering"),* for example, as follows.

[0204] A full length cDNA encoding an antibody molecule is prepared, and an appropriate length of a DNA fragment comprising a moiety encoding the antibody molecule is prepared.

[0205] A recombinant vector is prepared by inserting the DNA fragment or the full length cDNA into downstream of the promoter of an appropriate expression vector.

[0206] A transformant which produces the antibody molecule can be obtained by introducing the recombinant vector into a host cell suitable for the expression vector.

[0207] As the host cell, any of yeast, animal cell, insect cell, plant cell or the like can be used, so long as it can express the gene of interest.

[0208] A cell such as yeast, animal cell, insect cell, plant cell or the like into which an enzyme relating to the modification of an *N*-glycoside-linked sugar chain which binds to the Fc region of the antibody molecule is introduced by a genetic engineering technique can also be used as the host cell.

[0209] As the expression vector, a vector which is autonomously replicable in the host cell or can be integrated into the chromosome and comprises a promoter at such a position that the DNA encoding the antibody molecule of interest can be transferred is used.

[0210] The cDNA can be prepared from a human or non-human tissue or cell using, e.g., a probe primer specific for the antibody molecule of interest, in accordance with the methods described in the preparation of DNA in the item 1 (1)(a).

[0211] When a yeast is used as the host cell, examples of the expression vector include YEP13 (ATCC 37115), YEp24 (ATCC 37051), YCp50 (ATCC 37419) and the like.

[0212] Any promoter can be used, so long as it can function in yeast. Examples include a promoter of a gene of the glycolytic pathway such as a hexose kinase gene, *etc.,* PH05 promoter, PGK promoter, GAP promoter, ADH promoter, gal 1 promoter, gal 10 promoter, heat shock protein promoter, MF α1 promoter, CUP 1 promoter and the like.

[0213] Examples of the host cell include microorganisms belonging to the genus *Saccharomyces,* the genus *Schizosaccharomyces*, the genus *Kluyveromyces,* the genus *Trichosporon*, the genus *Schwanniomyces* and the like, such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Trichosporon pullulans* and *Schwanniomyces alluvius*, *etc.*

[0214] As the method for introducing the recombinant vector, any method can be used, so long as it can introduce DNA into yeast. Examples include electroporation *[Methods in Enzymology,* 194, 182 (1990)], spheroplast method [*Proc. Natl. Acad. Sci. USA*, 84, 1929 (1978)], lithium acetate method [*J. Bacteriol.*, 153, 163 (1983)], a method described in *Proc. Natl. Acad. Sci. USA*, 75, 1929 (1978) and the like.

[0215] When an animal cell is used as the host, examples of the expression vector include pcDNAI, pcDM8 (available from Funakoshi), pAGE107 [Japanese Published Examined Patent Application No. 22979/91; *Cytotechnology*, 3, 133 (1990)], pAS3-3 (Japanese Published Examined Patent Application No. 227075/90), pCDM8 [*Nature*, 329, 840 (1987)], pcDNAI/Amp (manufactured by Invitrogen), pREP4 (manufactured by Invitrogen), pAGE103 [*J. Biochemistry*, 101, 1307 (1987)], pAGE210 and the like.

[0216] Any promoter can be used, so long as it can function in an animal cell. Examples include a promoter of IE (immediate early) gene of cytomegalovirus (CMV), an early promoter of SV40, a promoter of retrovirus, a promoter of metallothionein, a heat shock promoter, an SRα promoter and the like. Also, an enhancer of the IE gene of human CMV may be used together with the promoter.

[0217] Examples of the host cell include a human cell such as Namalwa cell, a monkey cell such as COS cell, a Chinese hamster cell such as CHO cell or HBT5637 (Japanese Published Examined Patent Application No. 299/88), a rat myeloma cell, a mouse myeloma cell, a cell derived from syrian hamster kidney, an embryonic stem cell, a fertilized egg cell and the like.

[0218] As the method for introducing the recombinant vector, any method can be used, so long as it can introduce DNA into an animal cell. Examples include electroporation [*Cytotechnology*, 3, 133 (1990)], the calcium phosphate method (Japanese Published Examined Patent Application No. 227075/90), the lipofection method [*Proc. Natl. Acad.*